Co-Repressive Interaction and Clustering of Degrade-and-Fire Oscillators

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Strongly nonlinear degrade-and-fire (DF) oscillations may emerge in genetic circuits having a delayed negative feedback loop as their core element. Here we study the synchronization of DF oscillators coupled through a common repressor field. For weak coupling, initially distinct oscillators remain de-synchronized. For stronger coupling, oscillators can be forced to wait in the repressed state until the global repressor field is sufficiently degraded, and then they fire simultaneously forming a synchronized cluster. Our analytical theory provides necessary and sufficient conditions for clustering and specifies the maximum the number of clusters which can be formed in the asymptotic regime. We find that in the thermodynamic limit a phase transition occurs at a certain coupling strength from the weakly-clustered regime with only microscopic clusters to a strongly clustered regime when at least one giant cluster has to be present.

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Many gene regulatory networks contain negative feedback loops as their core elements [1]. The negative feedback provides robustness and noise-resistance to signaling pathways [2]. They also lie at the center of many natural rhythmic circuits (such as circadian clocks [3]) and synthetic gene oscillators [4–6]. In our recent work [7] we identified the delayed negative feedback as the mechanism of oscillations in the synthetic two-gene oscillator [5]. It is well known that delayed auto-repression can lead to oscillatory gene expression even with only a single regulatory element [8–12]. Typically, the period of delay-induced oscillations is comparable with the delay time and thus this mechanism seemingly could not explain rather slow oscillations observed in gene circuits. However, we showed that in a strongly nonlinear regime, the period of oscillations is determined by the rate of enzymatic degradation of the repressor protein and therefore can be arbitrarily longer than the transcriptional and translational delay. The essential mechanism of oscillations in this circuit is based on the periodic alternation of two regimes - slow degradation of repressor protein, and the following fast production of the repressor (firing), hence we termed these sawtooth-like oscillations "degrade-and-fire" in analogy with integrate-and-fire oscillations in neuronal circuits. Bacterial cells containing the DF circuit demonstrated robust oscillations, but due to cell-to-cell variability and stochastic effects, in the absence of cell-cell communication the bacterial colony quickly becomes desynchronized. In our subsequent work [13] we succeeded in synchronizing gene oscillators across bacterial colony, however the synchronized regime was achieved with a different circuit design that incorporated a quorum-sensing machinery producing small molecule AHL which served as signaling element necessary for cell-cell synchronization.

In this paper we study synchronization of DF oscillators through purely co-repressive interaction. To enable the analytical calculations, we replace the original nonlinear delay-differential model of the DF oscillator [7] by a piecewise linear model which assumes that the concentration x degrades from the maximum value (which can be scaled to 1) linearly with unit rate, $\dot{x} = -1$ (mimicking enzymatic decay with high enzyme affinity), until it it reaches the (small) threshold value $\eta > 0$, after which repressor concentration x is immediately reset to 1 ("fire"), and the process repeats. It is easy to see that the protein concentration oscillates with period $1 - \eta$.

Now we consider a population of N degrade-and-fire (DF) oscillators coupled through a common repressor field. We postulate that the dynamics of the concentration of repressor protein x_i is given by the following rules

- if positive, x_i degrades linearly with rate 1, *i.e.* $\dot{x}_i = -1$, or remains constant if it has reached 0,
- when the locally averaged concentration $\chi_i(t) = (1-\epsilon)x_i(t) + \epsilon X(t)$ (where $X(t) = \frac{1}{N}\sum_{i=1}^{N} x_i(t)$ and $0 \leqslant \epsilon \leqslant 1$ is the coupling strength parameter) reaches threshold η, the ith oscillator fires, and its concentration is reset to 1, *i.e.* $x_i(t+) = 1$.

With these simple evolution rules, one can readily see that all oscillators must fire indefinitely (*i.e.* there cannot be "oscillator death" regime for any of the oscillators). Indeed, starting from an arbitrary configuration $\{x_i\}_{i=1}^{N}$ for which all $\chi_i > \eta$, all concentrations decay towards 0 with time (those that have reached $x_i = 0$, remain at zero) and so do all χ_i. Thus the oscillator with the lowest x_i (possibly, more than one if several oscillators have identical concentrations x_i) eventually fires when the corresponding χ_i reaches η. After that, the oscillator j with new lowest x_j has to fire when its χ_j reaches η, and so on [14]. It is also clear that if any two oscillators in a population are in sync at certain time t_*, i.e. $x_i = x_j$, they will remain in sync for all $t > t_*$. What is not obvious however, is under which conditions oscillators that are initially out of sync will synchronize in the course

of the dynamics, and what the properties of the resulting clusters are. To answer these questions, we begin with the simple case of two oscillators.

Interaction of two DF oscillators. In this case oscillator i ($i = 1, 2$) fires when $\chi_i = (1-\epsilon/2)x_i + \epsilon x_{3-i}/2 = \eta$. Without loss of generality, we can assume that initially, one oscillator has just fired (*i.e.* $(x_1, x_2) = (x, 1)$ with $\eta < x \leqslant 1$) and denote $t_f(x) > 0$ the firing time associated with this configuration. The dynamics can be described through the return map R between ordered concentration pairs just after firings, $(x, 1) \mapsto R(x, 1) := (1 - t_f(x), 1)$.

The 2-dimensional dynamics of two DF oscillators is contained in a parameter dependent subset of the unit square $[0, 1]^2$ (see Fig. 1). For $\epsilon = 0$, firing occurs when either of the individual concentrations $x_i = \chi_i$ touches the threshold η. A simple computation shows that $R^2(x, 1) = (x, 1)$ for all x, *i.e.* we have a continuum of periodic orbits equivalent to the rigid rotation on a 2-dimensional torus (see Fig. 1a).

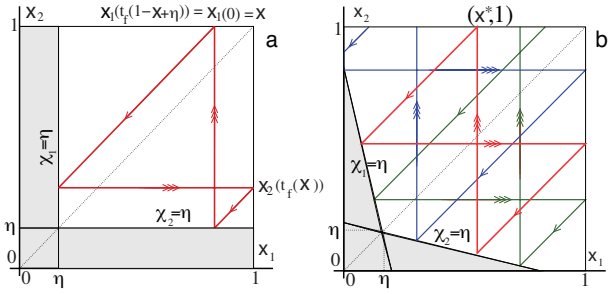

FIG. 1: Typical phase trajectories of two coupled DF oscillators. Simple arrows indicate motions between firings when both concentrations decay with rate 1. Triple arrows denote firings when the trajectory jumps from critical line $\chi_i = \eta$ to $x_i = 1$. (a) $\epsilon = 0$, every trajectory is periodic. (b) $\epsilon > 0$, for every initial condition except $x_1 = x_2$ the system asymptotically converges to a unique periodic trajectory passing through $(x^*, 1)$ where $x^* = 1 - 2(1-\eta)/(4-\epsilon)$ (thick red line).

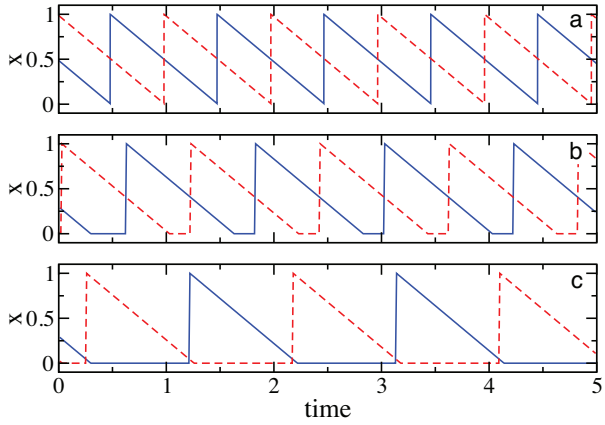

FIG. 2: Typical time series of two coupled DF oscillators for $\eta = 0.01$ and different values of ϵ: (a) $\epsilon = 0.01$; (b) $\epsilon = 0.05$; (c) $\epsilon = 0.5$

For $\epsilon > 0$, the dynamics changes drastically, as expected. Instead of a continuum of neutral periodic orbits, a single stable periodic orbit emerges which attracts all trajectories, except for the unstable periodic orbit lying on the diagonal $x_1 = x_2$ (see Fig. 1b). These are direct consequences of Lemmas 2 and 3 below. Furthermore, it can be shown that a unique and globally stable periodic orbit exists in arbitrary systems of two coupled DF oscillators with any monotonous degradation of both concentrations. Note that this result implies a somewhat unexpected corollary that two initially distinct oscillators never synchronize, and always remain distinct. Figure 2 shows typical trajectories of two coupled oscillators for $\eta = 0.01$ and different values of ϵ. As seen from the Figure, the two oscillators in the asymptotic regime are always in anti-phase, and, for large ϵ, one oscillator remains repressed ($x_i = 0$) almost the entire time when the other repressor is present (more precisely, it fires when the other concentration reaches the small value $2\eta/\epsilon$), and *vice versa*.

Many coupled DF oscillators. Now we turn to the analysis of the clustering dynamics of a population of N corepressively coupled DF oscillators. Before we proceed with analytical results, we illustrate the typical behavior of the system numerically. Figure 3 shows the time series of 50 oscillators with initially distinct values of x for $\eta = 0.01$ and different values of the coupling parameter ϵ. For small $\epsilon = 0.01$ (Fig. 3a), all oscillators fire before they ever reach zero, and so they remain distinct (no clustering). For larger $\epsilon = 0.03$ (Fig. 3b) some oscillators reach zero, and some of them (not all) synchronize, and thus asymptotically, only 8 clusters remain. Note that this number is not universal, as it depends on specific initial conditions. For $\epsilon = 0.05$ (Fig. 3c), only two clusters remain. Their "weights" however are not equal, in this example one cluster contains 24 oscillators, and another 26 oscillators. Again, this weight distribution is non-universal. And for very large $\epsilon = 0.5$ (Fig. 3d) oscillators produce repressor protein in almost exact anti-phase, one one repressor is present, the other one is absent.

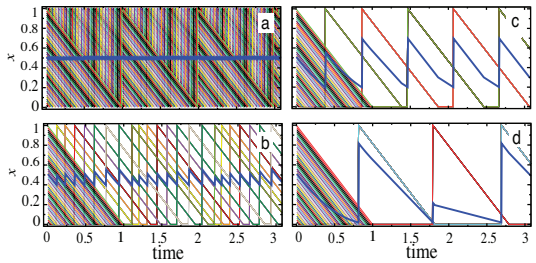

FIG. 3: Typical time series of 50 coupled DF oscillators and of their mean value $X(t)$ (thick/blue line) for $\eta = 0.01$ and different values of ϵ: (a) 0.01; (b) 0.03; (c) 0.05; (d) 0.5.

By grouping oscillators with identical value of x_i into one cluster, the population dynamics can be described via $\{(n_k, x_k)\}_{k=1}^{K}$ where $n_k \in \{1, ..., N\}$ denotes the size of the cluster k and x_k the corresponding repressor concentration ($K \geqslant N$ is the total number of clusters). In this viewpoint, the cluster size distribution $\{n_k\}$ obviously remains unaffected in time unless two clusters k

and k' fire together.

As before, we consider the return map between consecutive firings. Any ordering in $\{(n_k, x_k)\}$ is irrelevant because of the permutation symmetry in this system. However, it is more convenient to deal with ordered values of x_k. Thus we assume that $0 < x_1 < x_2 < \cdots < x_{K-1} < x_K = 1$. Given $\{n_k\}$, this defines the state configuration set where the return map $R \equiv R_{\{n_k\}}$ is effectively defined, *viz.* $\mathcal{T}_{\{n_k\}} = \{(x_1, ..., x_K) : 0 < x_1 < \cdots < x_K = 1$ and $(1-\epsilon)x_1 + \frac{\epsilon}{N}\sum_{k=1}^{K} n_k x_k > \eta\Big\}$. In order to maintain the ordering of protein concentrations in time, we must include cyclic permutations of indices in the return map dynamics. The absence of clustering requires that, when starting in $\mathcal{T}_{\{n_k\}}$, the after-firing configuration has to lie in $\mathcal{T}_{\{n_{k+1}\}}$ (where all indices are understood mod K) and this should hold for each $k = 1, ..., K_{\rm per}$ where $K_{\rm per}$ is the minimal size distribution period. This property globally holds in state configuration sets provided that the coupling strength is small enough.

Lemma 1 *$R_{\{n_k\}}\mathcal{T}_{\{n_k\}} \subset \mathcal{T}_{\{n_{k+1}\}}$ for all $k = 1, ...K_{per}$ iff*

$$\epsilon_\eta \leqslant \min_{k=1,...,K_{per}} \frac{N}{N - n_k - n_{k+1}} \text{ where } \epsilon_\eta := \epsilon/\eta \quad (1)$$

In particular, for $\epsilon_\eta < N/(N-2)$ which is the minimal RHS in (1), no clustering can ever occur, independently of the initial configuration. Notice that $N/(N-2) > 1$ and $\to 1$ as $N \to \infty$. Lemma 1 is rigorously proven in [15], but it can be intuitively understood as follows. The only way two clusters can merge is when one cluster reaches zero (collapse) and remains at zero at least until the following cluster also reaches zero. It is easy to see that in the worst case scenario two clusters 1 and 2 should be very close to zero when the other $K-2$ clusters are very close to 1. In the limit $x_{1,2} \to 0, x_3, ..., x_K \to 1$, after x_1 has collapsed, χ_1 reaches η when the value of x_2 is equal to $[N - \epsilon_\eta(N - n_1 - n_2)]/(N\epsilon_\eta)$, and in order to avoid merging of n_1 and n_2, this value has to be positive. Of course, the same condition has to be satisfied for all consecutive pairs n_k, n_{k+1}, hence the minimum in inequality (1).

Independently of (1), we may ask about the fate of the trajectories for which clustering occurs. Since the sequence of merging events is always finite, this actually would determine every possible asymptotic regime. In absence of clustering, the return map in $\mathcal{T}_{\{n_k\}}$ becomes $R^*_{\{n_k\}}(x_1, ..., x_K) := (x_2 - t_f, ..., x_K - t_f, 1)$ where t_f still denotes the firing time. (This map is actually $K-1$-dimensional because $(R^*_{\{n_k\}}x)_K = x_K = 1$.) Since the image $R^*_{\{n_k\}}x$ belongs to $\mathcal{T}_{\{n_{k+1}\}}$, one needs to iterate further until the point returns back to $\mathcal{T}_{\{n_k\}}$; hence the dynamics to study is the composed map $R^*_{\{n_{k+K_{\rm per}-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$. It can be shown [15] that this map is always a pure contraction.

Lemma 2 *For every $\{n_k\}_{k=1}^K$ and $0 < \epsilon \leqslant 1$, there is a norm in $\mathbb{R}^{K-1}$ for which $R^*_{\{n_{k+K_{per}-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$ is a global contraction.*

It follows that, in absence of clustering, every trajectory initially in $\mathcal{T}_{\{n_k\}}$ must approach a unique periodic orbit (whose single element in $\mathcal{T}_{\{n_k\}}$ is the fixed point of the composed map). More generally, every trajectory in a population of N oscillators must converge to the periodic orbit associated with some $\{n_k\}$. Hence, the asymptotic cluster size distributions and periodic orbits are in one-to-one correspondence. Accordingly, to get asymptotically attainable cluster distributions, it suffices to compute the existence domains of the corresponding periodic orbits. These domains are given by the following statement.

Lemma 3 *The periodic orbit in $\mathcal{T}_{\{n_k\}}$ exists iff*

$$\epsilon_\eta < \zeta(\{n_k\}) := \frac{2N^2}{N^2 - \sum_k n_k^2} \frac{1}{1 - \min_k \frac{n_k}{N - n_{k+1}}} \quad (2)$$

This claim is proved in [15] where we also give the explicit expressions for the periodic orbit. Note that when $n_K = N - n_1$ (*i.e.* $K = 2$), we have $\zeta(\{n_k\}) = +\infty$, which means that every two-cluster periodic orbit exists for any ϵ. In particular, for $n_1 = n_2 = 1$ this observation implies the result for two coupled oscillators presented above.

For $K > 2$, the critical value $\zeta(\{n_k\})$ is minimal for the (equi-)distribution where all $n_k = 1$ (in this case $K = N$) and the corresponding minimum is $\zeta_c = 2N/(N-2)$. So all possible periodic orbits exist for ϵ_η up to ζ_c. For larger ϵ_η, some periodic orbits disappear - certainly the one associated with equi-distribution - and the number of clusters K must eventually be less than N.

A closer look at the formula (2) reveals that when $\{n_k\}$ is only composed of microscopic clusters, *i.e.* when $\sum_k n_k^2 = \mathcal{O}(N)$, the corresponding critical value $\zeta(\{n_k\})$ approaches 2 in the thermodynamic limit $N \to \infty$. Therefore, such distributions do not perdure asymptotically beyond $\epsilon_\eta \simeq 2$ when N is large, and at least some of the clusters size n_k reach $\mathcal{O}(N)$ in the course of time. Moreover, distributions that contain macroscopic cluster(s) (*i.e.* $n_k \simeq \rho_k N$ with $\rho_k > 0$ for some k) all have critical value $\zeta(\{n_k\})$ larger than 2 in the thermodynamic limit. Thus, there is a sharp transition at a certain $\epsilon_c = \zeta_c \eta \to 2\eta$ in the system for large N, from a dynamical regime in which all kinds of cluster size distributions may exist asymptotically, to a regime where every asymptotic distribution contains at least one giant $\mathcal{O}(N)$ cluster.

Maximal number of clusters. The transition at $\epsilon_\eta = \zeta_c$ can be quantitatively described by estimating the number of asymptotic clusters as a function of ϵ_η. Since the precise number actually depends on the initial condition and can be as little as 2, the appropriate quantity to compute is the maximal possible number $K_{\max}$. It can be proven [15] that for any given $K \geqslant 3$ and $N \geqslant K$ (and $N \neq 6$ if $K = 3$), the critical value $\zeta(\{n_k\}_{k=1}^K)$ in (2) reaches its maximum for the distribution consisting of $K-1$ unitary clusters of weight $n_k = 1$ and one big cluster $n_K = N - K + 1$. In this case, we have

$$\zeta(\{n_k\}) = \zeta_{\max}(K) = \frac{2N^2(N-1)}{(K-1)(2N-K)(N-2)}$$

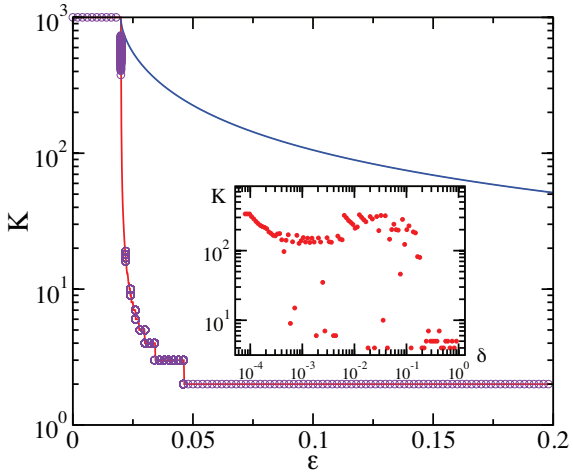

FIG. 4: Number of clusters in the asymptotic regime for 1000 coupled oscillators with $\eta = 0.01$ and 1000 different random initial conditions for each ϵ. The solid blue line indicates the upper bound (3), and the solid red line shows the number of clusters $K_e(\epsilon)$ for the uniform initial distribution. *Inset:* the number of clusters as a function of the initial distribution width δ for $\epsilon = 0.03$

and therefore all distributions with K clusters exist for $0 \leqslant \epsilon_\eta < \zeta_{\max}$. This immediately yields the maximum number of clusters at a given N and ϵ_η:

$$\begin{aligned} K_{\max}(\epsilon_\eta) &= N \text{ if } \epsilon_\eta < \zeta_c \\ &= N - \left\lfloor \frac{1}{2} + \sqrt{\frac{1}{4} + N(N-1)\left(1 - \frac{\zeta_c}{\epsilon_\eta}\right)} \right\rfloor \\ &\qquad\qquad \text{if } \zeta_c \leqslant \epsilon_\eta < \zeta_{\max} \quad (3) \\ &= 2 \text{ if } \epsilon_\eta \geqslant \zeta_{\max} \end{aligned} \tag{3}$$

This result indicates that a kind of second order phase transition takes place at $\epsilon_\eta = \zeta_c$ with $K_{\max}$ that behaves like $N(1-\sqrt{1-\zeta_c/\epsilon_\eta})$ slightly above the threshold. Formula (3) implies that for each $1 \leqslant K \leqslant K_{\max}(\epsilon_\eta)$, there exists a non-empty set of initial conditions which produces a stable periodic trajectory with K clusters. However, the "typical" number of clusters emerging from an arbitrary set of initial conditions can be much less than this upper bound. We performed numerical simulations of the full model with initial conditions randomly selected from the $[0,1]^N$ hypercube. Figure 4 shows that the typical number of clusters for a given ϵ can be well approximated by the number K_e corresponding to the uniformly distributed initial condition $x_i = i/N$. However, the number of asymptotic clusters depends strongly on the width of the distribution of initial states. We performed simulations for initial conditions x_i equi-distributed between $1-\delta$ and 1, and found that the number of asymptotic clusters strongly increases as the width of the initial distribution δ gets smaller, and approaches the values close to the upper bound $K_{\max}$ (Fig. 4, inset).

In summary, we presented an analytically solvable model of co-repressive coupling of degrade-and-fire oscillators in a strongly nonlinear regime. At a certain coupling strength this model exhibits a phase transition from a regime when all cluster distributions including non-clustered state can be attained to the regime of strong clustering characterized by the appearance of at least one giant $\mathcal{O}(N)$ cluster. Our model neglected the variability in parameters of individual oscillators and possible delays in the coupling. We plan to address these effects in our future work. The experimental realization of coupled gene oscillators [13] incorporated a different (co-excitatory) mechanism of coupling through a quorum-sensing mechanism. However, we believe that co-repressive coupling may also be realized experimentally, if for example oscillators produce an enzyme that degrade a freely diffusing inducer or the quorum sensing molecule activates a repressor protein. Our results demonstrate that a strong clustering can be expected in this case, and large out-of-phase clusters are likely to emerge.

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[14] Strictly speaking, we need an additional mild restriction on the coupling strength, namely $\epsilon < \frac{1-\eta}{1-1/N}$, in order to guarantee that firings reset every χ_i to values above η.
[15] Supplementary Information supplied by EPAPS.